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DEVOLPMENT AND VALIDATION OF ANALYTICAL METHOD FOR CITICOLINE AND PIRACETAM IN PHARMACEUTICAL DOSAGE FORM BY UV SPECTROPHOTOMETRIC METHOD

Mayur G. Prajapati*, Rajesh R. Parmar, Vishnu M. Patel, Dushyant A. Shah

Department of Quality Assurance, APMC College of Pharmaceutical Education and Research, College campus, Motipura, Himatnagar-383001, Gujarat-INDIA.

Keywords:

Citicoline(CITI), Piracetam(PIRA), UV Spectrophotometric, Absorption correction Method,Q-absorbance Method

For Correspondence:

Mayur G. Prajapati

Department of Quality Assurance, APMC College of Pharmaceutical Education and Research, College campus, Motipura, Himatnagar-383001, Gujarat-INDIA.

E-mail:

mayur8789@gmail.com

ABSTRACT

A Versatile, accurate, precise and economic method for simultaneous determination of Citicoline (CITI) and Piracetam (PIRA) in fixed dose combination products was developed. Method-1: absorption correction method and Method-2: Q-absorbance method. for method 1 Wavelengths selected for CITI was 266 nm as PIRA shows zero absorbance. So absorbance of PIRA was found by subtracting absorbance of CITI. Absorbance corrected for PIRA was measured at 226.5 nm.Method-2:by using wavelength 220 nm(λ max of PIRA)and 228 nm(isoabsorptive point) This method obeyed Beer's law in the concentration range of mixture of 10–50 µg/ml for CITI and 100-500 µg/ml for PIRA. The results of analysIs have been validated for linearity, accuracy and precision, LOD and LOQ of the proposed method.

INTRODUCTION

Citicoline (Cytidine-5'-diphosphocholine or CDP-choline) is a complex organic molecule that functions as an intermediate in the biosynthesis of phosphatidylcholine and chemically it is Cytidine 5'-{trihydrogen diphosphate} p'-[2-{trimethylammonio}ethyl] ester inner salt CDP-choline belongs to the group of biomolecules in living systems known as "nucleotides" that play important roles in cellular metabolism. Citicoline is readily absorbed in the GI tract and widely distributed throughout the body, crosses the blood-brain barrier and reaches the central nervous system (CNS), where it is incorporated into the membrane and microsomal phospholipid fraction. Citicoline activates biosynthesis of structural phospholipids of neuronal membranes, increases brain metabolism, and acts upon the levels of different neurotransmitters . It has a variety of beneficial effects in CNS injury models and cognitive enhancing, neuroprotective, and neurological disorders of the brain such as stroke, brain, trauma, Alzheimer's and Parkinson's disease. Piracetam is a chemically 2-(2-Oxopyrrolidin-1-yl)acetamide .Piracetam's mechanism of action, as with racetams in general, is not fully understood. The drug influences neuronal and vascular functions and influences cognitive function without acting as a sedative or stimulant. Piracetam is a positive allosteric modulator of the AMPA receptor. Piracetam improves the function of the neurotransmitter acetylcholine via muscarinic cholinergic (ACh) receptors which are implicated in memory processes.Furthermore, Piracetam may have an effect on NMDA glutamate receptors, which are involved with learning and memory processes. Piracetam is thought to increase cell membrane permeability. Piracetam may exert its global effect on brain neurotransmission via modulation of ion channels (*i.e.*, Na^+, K^+). It has been found to increase oxygen consumption in the brain, apparently in connection to ATP metabolism, and increases the activity of adenylate kinase in rat brains. Citicoline is not official in any Pharmacopeia and Piracetam is official in British Pharmacopoeia. A survey of literature revealed that no chromatographic and Spectrophotometric methods are reported for determination Citicoline and Piracetam in combined dosage form. The present work describes simple, precise, accurate and economical spectrophotometric method have been developed for simultaneous estimation of Citicoline and Piracetam in combined dosage form.

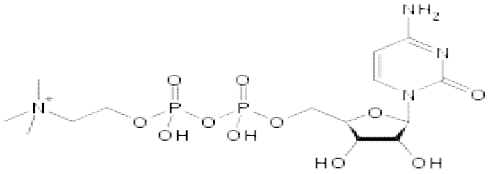

Figure-1 Citicoline

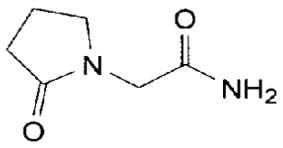

Figure-2 Piracetam

MATERIAL AND METHOD

Instrument

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions.

Reagents and Chemicals

Reference Standards of CITICOLINE and PIRACETAM were obtained as gift samples from the Montej laboratories. The drug sample (tablets) PREXARON PLUS manufactured by Intas Pharmaceuticals, Sikkim, were procured from market. All other reagents were of analytical grade for Spectrophotometric method.

Procedures

Preparation of Standard Stock Solution and Calibration curve:

A mixed stock solution of CITI (1000 µg/ml) and PIRA (1000 µg/ml) was prepared by accurately weighing CITI (100 mg) and PIRA (100 mg), dissolving in distilled water and diluted to 100ml with the same solvent in the same volumetric flask. Then further dilution with 0.1 N NaOH and again dilutions were made as such that five solutions prepared containing 10-50µg/ml CITI and 100-500µg/ml PIRA. Calibration curve were prepared for CITI using absorbance of mixture at 266nm and for PIRA using corrected absorbance at 226.5nm.

Method 1:Absorption correction method

Absorbance spectrum of pure CITI was scanned in the spectrum basic mode. Using the cursor function, the absorbance corresponding to 266 nm (wavelength λ1, the wavelength of minimum absorbance for CITI) was noted from spectrum. Then the cursor function was moved along with peak curve until the absorbance equal to that of absorbance at 266 nm was found. The wavelength obtain corresponding to this absorbance value was 226.5 nm (λ2). The absorbance of various dilutions of CITI in water + 0.1N NaOH was measured at 266 nm. Absorbance spectrum of pure PIRA was also scanned in the spectrum basic mode. PIRA showed some absorbance value at 226.5 (λ2) while it does not show any absorbance value at 266 nm. The absorbance value at 266 nm is due to CITI only in the combined mixture of both drugs. Wavelength λ1 (266 nm) was selected for the measurement of CITI and for measurement of PIRA corrected absorbance by subtraction of absorbance of mixture at λ_2.

Method 2:Q-absorbance Method

The absorbance ratio method of analysis is based on the absorbance at two selected wavelengths; one is an isosbestic point and the other being the wavelength of maximum

absorption of one of the two components. From overlain spectra (Figure-1) wavelength 228 nm (isosbestic point) and 220 nm (λ max of Piracetam) are selected for Q-Absorbance equation (3 & 4).

Cx = (Qm-Qy) x A1/(Qx-Qy) x ax1 ……(3)

Cy = (Qm-Qx) x A1/(Qy-Qx) x ay1 ……(4)

Where A1 and A2 are absorbance of sample solution at 228 nm and 220 nm respectively. ax1 = Absorptivity of citicoline at 228 nm, ax_2= Absorptivity of citicoline at 220 nm,

ay_1= Absorptivity of piracetam at 228 nm

ay_2= Absorptivity of piracetam at 220 nm

C_X and C_Y are concentration of citicoline and piracetam in sample solution.

Procedure for tablet formulation

Twenty tablets were accurately weighed and crushed. Average weight of the content per tablet was calculated. A quantity of tablet powder equivalent to 500mg of Citicoline and 800mg of Piracetam was transferred to 100 ml volumetric flask and dissolved in Distilled water , was then filtered through whatman filter. 10 ml of filtrate was further diluted with a 0.1 N NaOH to get a concentration of about 500µg/ml CITI and 800µg/ml of PIRA, then again pipette out 5 ml of filtrate and diluted with a 0.1 N NaOH to get a concentration of 25 µg/ml CITI and 40 µg/ml PIRA. For PIRA, to get in a concentration range standard addition of 2 ml of stock solution(1000 µg/ml) of piracetam is required.The absorbance of sample solution was measured at 266nm and 226.5nm in 1cm cell against the blank.

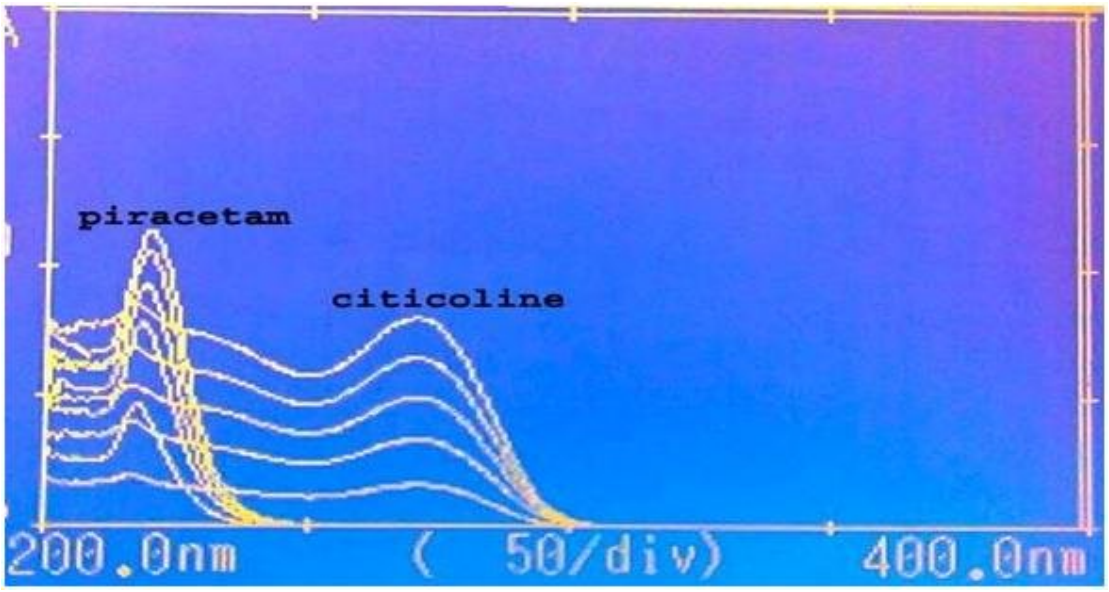

Figure-3 Overlain spectra of citicoline (10-50µg/ml) and piracetam (100-500µg/ml)

Table-1 Optical Characteristic:

Method I Absorption correction method		
Parameters	**CITICOLINE**	**PIRACETAM**
Wavelength (nm)	266	226.5
Beer's law limit (µg /ml)	10-50	100-500
Regression equation (y = a + bc)	y = 0.015x + 0.038	y = 0.001x + 0.087
Slope (b)	0.015328	0.001079
Intercept (a)	0.038811	0.087467
Correlation coefficient (r^2)	0.999873	0.999068
LOD (µg/ml)	0.269645	7.1242
LOQ (µg /ml)	0.8171	21.5885
Precision(% RSD,n=3) Interday Intraday	 0.4666 0.4372	 0.8412 0.8295

Method II Q-ABSORBANCE METHOD				
Parameter	**CITICOLINE**		**PIRACETAM**	
Wavelength (nm)	228 nm	220 nm	228 nm	220 nm
Beer's law limit (µg /ml)	10-50	10-50	100-500	100-500
Regression equation (y = a + bc)	y = 0.017x -0.049	y = 0.017x -0.058	y = 0.001x +0.013	y = 0.001x +0.245
Slope (b)	0.017	0.017	0.001	0.001
Intercept (a)	0.049	0.058	0.013	0.245
Correlation coefficient (r^2)	0.998	0.998	0.998	0.998
LOD (µg/ml)	0.43	0.31	6.24	12.00
LOQ (µg /ml)	1.32	0.96	18.91	36.36
Precision(% RSD,n=3) Interday Intraday	0.5313 0.6128	0.4928 0.5734	0.6643 0.7518	0.7221 0.4613

*SD = Standard deviation ** RSD=Relative Standard deviation

Table-2 Results of the recovery studies

Method	Recovery Level	% Recovery	SD	% Recovery	SD
		Citicoline		Piracetam	
I	80%	100.13	±0.50	100.66	±0.87
	100%	100.13	±0.94	99.95	±0.38
	120%	100.8	±1.70	99.83	±0.87
II	80%	100.57	±0.48	100.19	±0.37
	100%	100.12	±0.79	100.01	±0.25
	120%	100.2	±0.23	100.44	±0.93

*SD = Standard deviation

Table-3 Results of analysis of tablet formulation

Drugs	Absorption correction method	Q-Absorbance method
	%Assay ± SD(n=6)	
Citicoline(500 mg)	100.5± 0.30	100.29±0.20
piracetam (800 mg)	100.14± 0.25	100.21 ± 0.35

Validation of the Method according to ICH Guidelines

Validation of the method was done according to ICH guidelines for Simultaneous Equation method.

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 10-50 µg/ml and 100-500 µg/ml for CITI and PIRA respectively. Accurately measured standard solutions of CITI (1, 2, 3, 4, and 5 ml) and PIRA (10,20,30,40 and 50 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with 0.1N NaOH.For method 1: The absorbances of the solutions were measured at 266 nm of CITI, 226.5 nm of PIRA and for method 2:The absorbance of solution were measured at 220 nm and 228nm(Isoabsorptive point) against 0.1N NaOH as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Precision (repeatability)

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration.

Intermediate precision (reproducibility):

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of CITI and PIRA.

Accuracy (recovery study):

To check the accuracy of the proposed methods, recovery studies carried out at 80%, 100%, and 120% of the test concentration as per ICH Guideline. The recovery study was performed three times at each level.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULT AND DISSCUSSION

The solubility of CITICOLINE(CITI) and PIRACETAM (PIRA) was studied and 0.1N NaOH was selected as a choice of solvent. For absorption correction method Citicoline and Piracetam showed well defined λmax at 266.0 nm and 226.5 nm respectively.For Q-absorbance method The two drugs also show an isoabsorptive wavelength at 228 nm, where both the drugs have same absorptivity value. The other wavelength is 220 nm(λmax of piracetam) was considered for development of Q-absorbance method.The two drugs individually and in their mixture were found to follow Beer-Lambert's law over the concentration range of 10-50 µg/ml and 100-500 µg/mL for CITI and PIRA respectively.

CONCLUSION

The proposed methods are simple, rapid and validated in terms of linearity, precision, accuracy, reproducibility, and can be used successfully for routine simultaneous estimation of citicoline and piracetam in pure and TABLET dosage forms.

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